

A NEW SPECIES OF THE GENUS *PEDETONTINUS* (MICROCORYPHIA, MACHILIDAE) FROM CHINA

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Abstract *Pedetontinus laoshanensis* sp. nov. is described from Jiangsu Province, China. It is characterized by the parameres of 1 + 6 divisions and the ratio of length to basal width of urosternite V about 2/3. It is closely related to *P. tianmuensis*, *P. taishanensis* and *P. wudangensis*, but differs in the ratio of length to width of compound eye, the number of division of gonapophysis, the extending position of penis and parameres, and the ratio of basal part to terminal part of penis. The updated key to the species of *Pedetontinus* in the world is provided. We also examined the inter-species relationships of Chinese *Pedetontinus* based on partial sequences of mitochondrial cytochrome c oxidase subunit I (CO I). CO I gene sequences using Kimura's two parameter model showed intraspecies genetic divergence (< 1.3 %) and interspecies divergence ranged from 9.6 % to 15.4 %. The molecular study further supported the species status of *P. laoshanensis* sp. nov.

Key words Archacognatha, Microcoryphia, *Pedetontinus*, new species, China, CO I.

1 Introduction

The genus *Pedetontinus* Silvestri, 1943 can be easily recognized by the absence of scales on antennal flagellum, 1 + 1 eversible vesicles present on abdominal segments I – VII, paired ocelli shoe-shaped. 17 species of the genus *Pedetontinus* Silvestri have been recorded from China, Japan and Korea (Silvestri, 1943; Mendes, 1990a, b; Xue and Yin, 1991; Choe and Lee, 2001a, b; Zhang *et al.*, 2005; Zhang and Li, 2009; Cheng *et al.*, 2011; Zhang and Zhou, 2011). Those species can be divided into three group, viz: “wide-eyed group” (*P. aureus* Choe and Lee, 2001; *P. lineatus* Choe and Lee, 2001; *P. luanchuanensis* Cheng *et al.*, 2011; *P. rhombus* Choe and Lee, 2001; *P. songi* Zhang and Li, 2009; *P. tianmuensis* Xue and Yin, 1991; *P. yinae* Zhang *et al.*, 2005; *P. jiuzaishensis* Zhang and Zhou, 2011; *P. maijiensis* Zhang and Zhou, 2011; *P. wudangensis* Zhang and Zhou, 2011), “long-eyed group” (*P. szeptyckii* Mendes, 1992) and “long-equal-wide-eyed group” (*P. ishii* Silvestri, 1943; *P. dicrocerus* Silvestri, 1943; *P. kurwanae* Silvestri, 1943; *P. esakii* Silvestri, 1943; *P. yosii* Silvestri, 1943; *P. taishanensis* Zhang and Zhou, 2011). To date, there are about 29 species of Archaeognatha found in China (Silvestri, 1943; Xue and Yin, 1991; Sturm and Machida, 2001; Huang *et al.*, 2006; Yu *et al.*, 2010; Deng *et al.*, 2011; Song *et al.*, 2011; Zhang and Zhou, 2011).

During the study of the bristletail specimens collected from Nanjing City, Jiangsu Province, China, one species of genus *Pedetontinus* was identified as new

to science and described in present paper. The updated key to the genus *Pedetontinus* in the world is also provided. We also examined the inter-species relationships of Chinese *Pedetontinus* based on partial sequences of mitochondrial cytochrome (CO I).

2 Materials and Methods

2.1 Morphology

All specimens were preserved in 95 % alcohol to prevent DNA degradation. Taxonomically important parts of species were dissected and mounted on slides in Tendeiro liquid. All materials were examined under a Nikon SMZ 1500 stereomicroscope or an Olympus BH2 compound microscope. The remaining samples were put in 1.5 ml plastic tubes with 100 % alcohol and stored at -20 °C for DNA extraction.

The specimens are deposited in the College of Chemistry and Life Science, Zhejiang Normal University, China. The morphological terminology follows that of Mendes (1990b). The measurements in the paper are in millimeters (mm).

2.2 DNA extraction, amplification and sequencing

DNA was extracted from the specimens using the DNeasy Blood and Tissue Kit (Qiagen, Hilden Germany). Purified DNA was amplified for CO I mitochondrial sequences via polymerase chain reaction (PCR) using primers CO I-F (5'-ACA AAT CAT AAG GAT ATT GG-3') and C1-N-2329 (5'-ACT GTA AAT ATA TGA TGT GCT CA-3') (Simon *et al.*, 1994; Zhang and Zhou, 2011). In the PCR 50-μl reaction volumes were used according to instruction.

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This research was supported by the National Natural Science Foundation of China Project (NSFC) (31000966).

Received 7 July 2012, accepted 28 Sep. 2012.

Initial denaturation (4 min at 94 °C) was followed by 35 cycles of 50 s at 94 °C, 50 s at 48 °C, 60 s at 72 °C, and a final extension at 72 °C for 10 min, using a MJ Mini thermal cycler (BioRad, USA). The PCR clean-up of PCR products was performed using an Axygen PCR clean-up kit (Axygen, Hangzhou, China); in cases where gel purification was necessary, the Axygen agarose-out kit (Axygen) was used. The purified DNA was sequenced in forward and reverse directions by ABI PRISM 377 or 3730.

2.3 Analysis of DNA sequences

The nucleotide data (744 bp) were aligned using CLUSTAL W in MEGA 5.0 (Tamura *et al.*, 2011). Neighbour joining analysis (NJ) and Maximum parsimony analysis (MP) was performed with PAUP* 4.0b10 (Swofford, 2002) using one thousand bootstrap replicates. Bayesian inference analysis (BI) was performed with MRBAYES 3.0B4 (Huelsenbeck and Ronquist, 2001), using the GTR + I + G model. Eight chains ran in parallel for 1 000 000 generations, sampling trees every 1 000 generations. Bayesian analyses were repeated twice, always retrieving the same topology. According to the likelihood plots, -lnL values stabilized with 20 000 generations, so the first 20 000 generations were discarded as burn-in, while Bayesian posterior probabilities were calculated according to the remaining set of trees. Model selection for the nucleotides alignment dataset was performed with Modeltest

version 3.7 (Posada and Crandall, 1998). The model of GTR + I + G was chosen for Bayesian analyses. The CO I gene information of *P. laoshanensis* has been assigned GenBank accession No. EU699175 – EU699177.

3 Results and Discussion

3.1 Molecular analysis

The total alignment of the sequenced portions of Chinese *Pedetontinus* mitochondrial CO I gene consist of 744 bp. There are 218 variable sites in the 744 bp character matrix (29.3 %). In most cases, the CO I gene sequences using MEGA 5.0 with Kimura's two parameter model (K2p) showed intraspecies genetic divergence (< 1.3 %) and interspecies genetic divergence ranged between 9.6 % (*P. wudangensis* versus *P. laoshanensis* sp. nov.) and 15.4 % (*P. wudangensis* versus *P. taishanensis*) (Table 1). The average interspecies divergence of *Pedetontinus* is 12.7 % and the average intraspecies genetic divergence of *Pedetontinus* is from 0 to 1.3 %.

Both the phylogenetic position of *P. laoshanensis* in the trees (BI, MP, and NJ) (Fig. 1) and the genetic distances between interspecies of *Pedetontinus* (Table 1) supported that *P. laoshanensis* is a new species. Considering both the morphological and molecular evidence, the new species, *P. laoshanensis* sp. nov., is new to science herein.

Table 1. Genetic diversities among interspecies of *Pedetontinus* based on CO I sequences using Kimura 2-parameter model.

Species of <i>Pedetontinus</i>	1	2	3	4	5	6	7	8	9
1 <i>P. luanchuanensis</i>									
2 <i>P. songi</i>	0.133								
3 <i>P. tianmuensis</i>	0.141	0.119							
4 <i>P. wudangensis</i>	0.127	0.129	0.093						
5 <i>P. maijiensis</i>	0.116	0.131	0.125	0.111					
6 <i>P. taishanensis</i>	0.140	0.138	0.137	0.154	0.147				
7 <i>P. juzhaiensis</i>	0.121	0.142	0.132	0.119	0.118	0.131			
8 <i>P. yinae</i>	0.136	0.134	0.110	0.106	0.128	0.136	0.109		
9 <i>P. laoshanensis</i> sp. nov.	0.121	0.129	0.097	0.096	0.119	0.140	0.104	0.109	

3.2 Taxonomy

Machilidae Grassi, 1888

Petrobiinae Paclt, 1970

***Pedetontinus laoshanensis* sp. nov.** (Figs 2 – 17)

Holotype ♂ (No. ZJNUSB050), under basalt rock covered with lichen or pine needle of Mt. Lao (32°07'N, 118°36'E; alt. 145 m), Nanjing City, Jiangsu Province, China, 1 Aug. 2004, collected by LI Peng and ZHANG Jia-Yong. Paratypes: 7 ♂♂ (No. ZJNUSB051 – 057), 8 ♀♀ (No. ZJNUSB058 – 065), same data as holotype; 5 ♂♂ (No. ZJNUSB066 – 070), 6 ♀♀ (No. ZJNUSB071 –

076), under rock with deadwood and defoliation of Mt. Qixia (32°09'N, 118°57'E; alt. 121 m), Nanjing City, Jiangsu Province, China, 15 May 2011, collected by LI Peng, YU Dan-Na and ZHANG Jia-Yong.

Males. Body length 7.0 – 8.0 mm ($n = 13$); antennae 5.8 – 6.0 mm; terminal filament 7.5 – 8.0 mm; cerci 2.9 – 3.3 mm. Body reddish brown brownish grey, covered densely with scales and with pigments. Terga mostly covered with brown scales. No black spots present on body side of terga VII. Epidermic pigment on head capsule, labium, labial

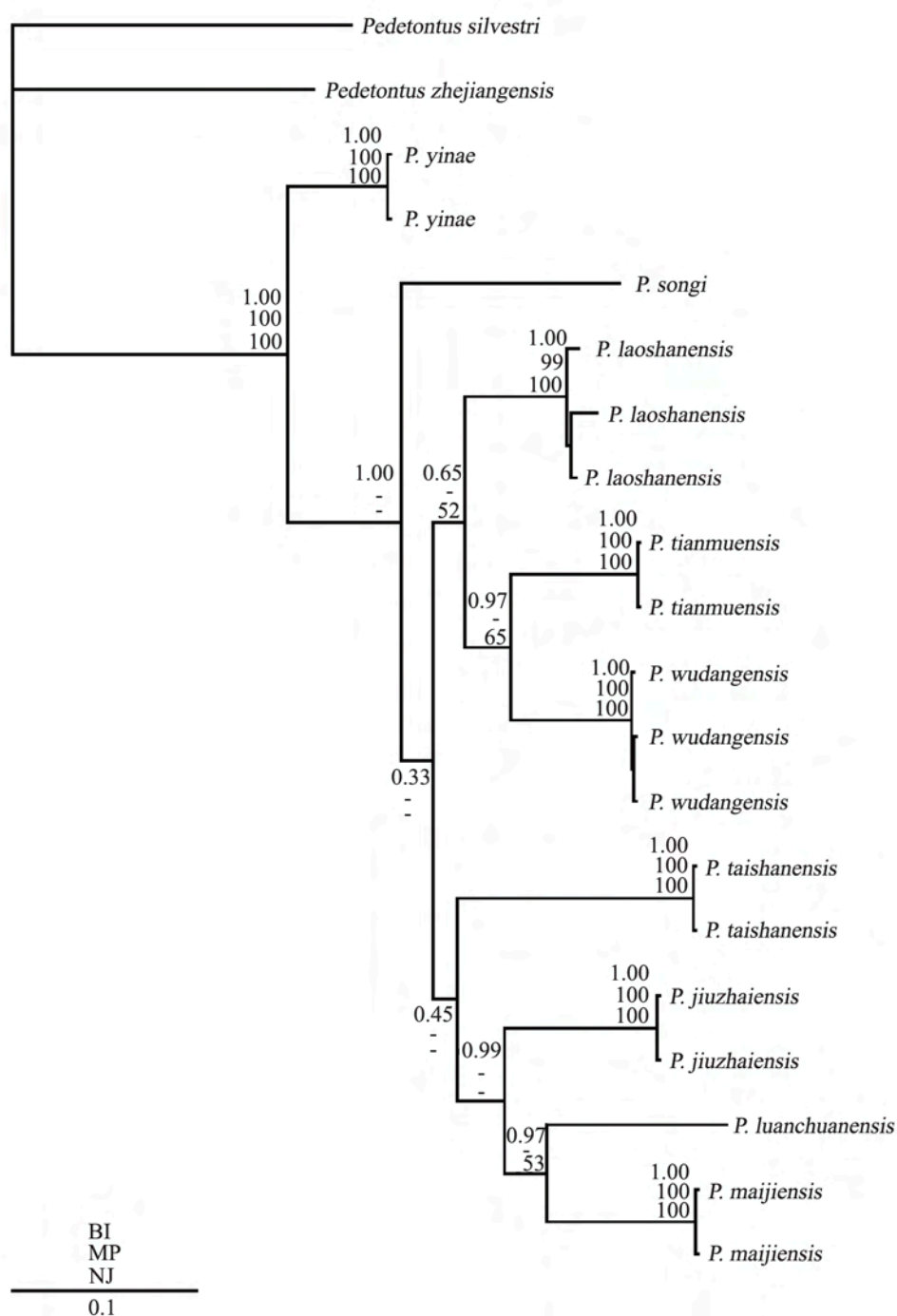


Fig. 1. Phylogenetic tree of the relationships among 11 bristletail species based on cytochrome oxidase subunit I (CO I) gene sequences. Branch lengths and topology are from the Bayesian analysis. Numbers above branches specify posterior probabilities from Bayesian inference (BI), maximum parsimony (MP, 1 000 replicates) and neighbour joining (NJ, 1 000 replicates) analyses, respectively.

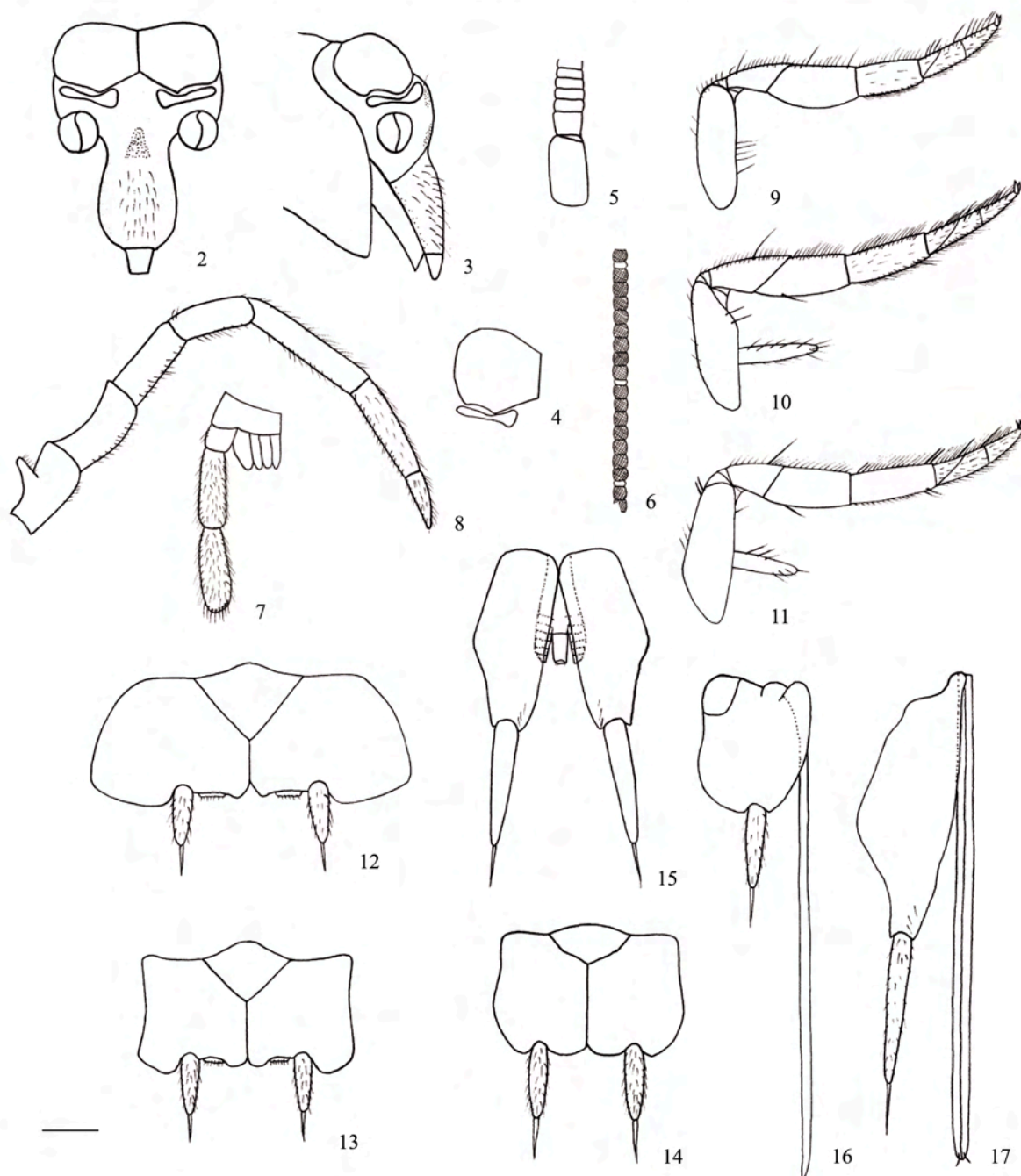
palp, mandibles, maxillae, maxillary palp.

Head brown between antennae, clypeus and labrum (Figs 2 – 3). Clypeus and labrum covered with numerous short, thin setae. Frons convex moderately, with two long setae.

Compound eyes round, large and black-brownish, with a small yellowish patch on inner corner (Fig. 2), ratio of contact line to length (cl/l) as 0.50 – 0.55; ratio of length to width (l/w) as 0.99 –

1.00. Paired ocelli shoe-shaped, enlarged in submedian area, reddish brown (in alcohol) (Fig. 4).

Antennae (Figs 5 – 6) shorter than body, ratio of antennae length to body length about 0.8 – 0.9. Scapus and pedicellus of antennae densely scaled, flagellum not scaled. Scapus rather long (ratio of length to width about 1.5); pedicellus length equal to width. Flagellum uniformly brownish, junctions



Figs 2 – 17. *Pedetontinus laoshanensis* sp. nov. 2 – 15. Male. 16 – 17. Female. 2. Head, frontal view, 3. Head, lateral view, 4. Oculus and ocellus. 5. Part of antenna with distal chain. 6. Part of antenna with scapus. 7. Labial palp. 8. Maxillary palp. 9. Fore leg. 10. Mid leg. 11. Hind leg. 12. Urosternite V. 13. Urosternite VII. 14. Coxite of the VIII abdominal segment. 15. Coxite of the IX abdominal segment with genital appendages. 16. Coxite of the VIII abdominal segment with anterior gonapophyses. 17. Coxite of the IX abdominal segment with posterior gonapophyses. Scale bar = 0.25 mm.

between segments light-grey. Maximum divisions of flagellum maximum observed as 22 – 23, proximal 9 divisions not annulated, divisions 10 – 14 annulated into 2 – 5 annuli, divisions 15 – 17 with 6 – 8 annuli, 18 – 23 with 9 annuli. The distal chains with 9 – 10 annuli, each one about as long as wide.

Labial palp as show in Fig. 7, article I without scaled and articles II – III sparsely scaled and with

dense setae; article III almost club like, with sensorial cones on ventral apical part.

Mandibles quite robust, with four typical apical teeth. Article I – VII of maxillary palp (Fig. 8) with setae on ventral surface. Setae in articles VI – VII denser than those in articles I – V. Article II – VI scaled densely, and article VII scaled sparsely. External apophysis of article I cone-shaped and slightly curved

backwards with setae sparsely on ventral surface. Article II with dense short setae on ventral surface, not extending beyond article III. Article III with dense short setae on ventral surface except center area without setae and two setae on internal distal end on dorsal surface. Article IV with dense short or long setae on ventral surface, one long setae on center ventral surface, and one long setae on internal distal end. Article V with dense setae on ventral surface and sparse setae on dorsal surface. Article VI – VII with dense setae, article VII cone-shaped. Dorsal surface of the articles V – VII with hyaline spines V as 2 – 4, VI as 12 – 14, VII as 10 – 14. Length ratio of article VII/VI as 0.60 – 0.63, IV/V as 0.68 – 0.72.

Legs and coxal stylets scaled (Figs 9 – 11). Mid and hind legs with coxal stylets (about 1/2 coxal length). Femur of fore leg not swollen, ratio of length to width as 2.0. One strong black spines present on femur, light-colored. Length of tibia I as 0.36 – 0.40 mm, tibia II as 0.40 mm, tibia III as 0.50 mm. Fore legs stronger than others, tibia of hind legs elongated.

Urosternites not pigmented. Abdominal stylets not especially elongated except segment IX. Abdominal segments I – VII with a pair of eversible vesicles. Sternum V with nearly right posterior angle ($87^\circ - 90^\circ$); ratio of length to basal width of sternum V as 0.70 (Fig. 12). Urosternite VII without swollen on its inner posterior part (Fig. 13). Urosternite VIII without eversible vesicles and parameres (Fig. 14).

Penis and parameres with 1 + 6 divisions (or 7 divisions) extending backward to 1/2 of length of the urosternite IX (Fig. 15). Penis as long as paramere, ratio of basal part to terminal part as 3.2, opening of penis small and apical. Male genitalia completely covered by urosternite IX. Apical spine of abdominal stylets strong, medium-sized. Coxite IX with 3 – 4 spines near apex. Length ratios of stylet (excluding apical spine) to coxite V as 0.45 – 0.50, VIII as 0.54 – 0.64, IX as 0.67 – 0.70. Length ratios of apical spine to stylet V as 0.50, VIII as 0.44 – 0.50, IX as 0.33 – 0.37.

Terminal filament and cerci with numerous scales and setae, but without piliform scales.

Females. Body length 8.1 – 9.0 mm ($n = 14$), antennae 5.8 – 6.8 mm, terminal filament 7.6 – 8.1 mm, cerci 3.1 – 4.0 mm. Scale pattern and epidermic pigment as in male.

Head as in male, with more setae present in the labrum and the clypeus than in male.

Compound eyes as in male, ratio of contact line to length (cl/l) as 0.50 – 0.55, ratio of length to width (l/w) as 0.99 – 1.00.

Antennae as in male. Length ratio of antennae length to body as 0.75 – 0.80. Scapus rather long

(ratio of length to width about 1.5). Maximum divisions of flagellum maximum observed as 23. The distal chains with 9 annuli, each one about 1.5 times long as wide.

Maxillary palp as in male. Articles II – VI densely scaled. Article I only scaled on ventral surface, VI – VII with dense setae and VII cone-shaped. Dorsal surface of articles V – VII with hyaline spines V as 1 – 3, VI as 14, VII as 11 – 12. Length ratio of article VII/V as 0.6, IV/V as 0.65 – 0.68.

Labial palp as in male.

Legs as in male. Femur of fore leg not swollen, ratio of length to width as 1.70. Spines present on femur, light-colored. Length of tibia I as 0.40 mm, tibia II 0.40 mm, tibia III 0.55 mm. Fore leg stronger than others, tibia of hind leg obviously elongated.

Abdominal segments as in male. Sternum V with nearly right posterior angle ($87^\circ - 90^\circ$); ratio of length to basal width of sternum V as 0.70. Medial part of urosternite VII projected and swollen, exceeding posterior margin.

Ovipositor robust, with tertiary type and slightly exceeding beyond the stylet of urosternite IX. Gonapophysis VIII with 40 – 43 divisions, sensilla present except 3 – 4 basal divisions (Fig. 16). Gonapophysis IX with 38 – 42 divisions, the basal 20 – 24 divisions with some short setae (Fig. 17). Coxite IX with 3 spines near apex. Length ratios of stylet (excluding apical spine) to coxite V as 0.50, VIII as 0.68, IX as 0.65. Length ratios of apical spine to stylet V as 0.50 – 0.60, VIII as 0.47 – 0.50, IX as 0.28 – 0.35.

Terminal filaments and cerci similar to those of male.

Diagnosis. *P. laoshanensis* sp. nov. belongs to “long-equal-wide-eyed group”, with parameres of 1 + 6 divisions, penis and paramere extending backward to 1/2 length of coxite IX, ratio of basal part to terminal part of penis 2.4, contact line between oculi slightly shorter than 2/3 length of ocular. *P. laoshanensis* sp. nov. is most closely related to *P. taishanensis*, *P. tianmuensis* and *P. wudangensis*, but differs in the ratio of length to width of compound eye, the number of division of gonapophysis, the extending position of penis and parameres, and the ratio of basal part to terminal part of penis. The new species is easily distinguished from other known *Pedetontinus* species in China and its vicinities as following: the pigmentation of whole body, color pattern, ratio of length to width of eyes, the number of setae of maxillary palp.

Etymology. The specific name refers to the type locality Mt. Lao.

Distribution. China (Jiangsu Province).

The updated key to species of *Pedetontinus* in the world.

1. Compound eye normal, length as long as width, or length longer than width 2
Compound eye width longer than length 9
2. Compound eye length longer than width, legs with hyaline spines, transparent spiniform seta in legs, penis shorter than paramere *P. szeptyckii*
Compound eye length as long as width 3
3. Boundary line between oculi as long as, or longer than 2/3 length of ocular 4
Boundary line between oculi shorter than 2/3 length of ocular 7
4. Ratio of length to basal width of urosternite V greater than 2/3, a pair of paramere with 1 + 7 articles *P. ishii*
Ratio of length to basal width of urosternite V as long as, or less than 2/3 5
5. Ratio of length to basal width of urosternite V as long as 2/3, a pair of paramere with 1 + 6 articles *P. taishanensis*
Ratio of length to basal width of urosternite V less than 2/3 6
6. Ratio of length to basal width of urosternite V about 3/8, the terminal segments of flagellum with 6 or 7 annuli *P. dicrocerus*
Ratio of length to basal width of urosternite V about 1/4, the terminal segments of flagellum with 7 or 8 annuli in female, 9 annuli in male *P. kuwanai*
7. Ratio of length to basal width of urosternite V less than 1/2 8
Ratio of length to basal width of urosternite V about 1/2, a pair of paramere with 1 + 6 articles *P. laoshanensis* sp. nov.
8. Ratio of length to basal width of urosternite V about 1/3, the terminal segments of flagellum with 6 or 7 annuli in female, 9 or 10 annuli in male *P. esakii*
Ratio of length to basal width of urosternite V about 1/4, the terminal segments of flagellum with 7 or 8 annuli in female, 9 annuli in male *P. yosii*
9. Boundary line between oculi as long as, or longer than 2/3 length of ocular 10
Boundary line between oculi slightly shorter than 2/3 length of ocular 15
10. Boundary line between oculi as long as 2/3 of ocular length 11
Boundary line between oculi longer than 2/3 length of ocular 12
11. Penis slightly longer than paramere and extending backward to 2/3 length of coxite IX 13
Penis as long as paramere, parameres with 1 + 6 divisions *P. wudangensis*
12. Parameres with 1 + 5 divisions *P. aureus*
Parameres with 1 + 5 articles *P. songi*
13. Paramere with 1 + 6 articles 14
Paramere with 1 + 5 articles, the ratio of length to basal width of urosternite V less than 1/2 *P. lineatus*
14. Body length about 11 – 14 mm, gonapophyses with 51 – 58 divisions, color of antenna uniformity *P. rhombeus*
Body length about 7 – 8 mm, gonapophyses with 41 – 45 divisions, proximal half of flagellum whitish in color, others uniformity *P. yinai*
15. Penis and paramere extending backward to 2/3 length of coxite IX 16
Penis and paramere extending backward to 3/4 length of coxite IX, paramere with 1 + 6 articles *P. tianmuensis*
16. Paramere with 1 + 5 articles 17
Paramere with 1 + 4 articles *P. majiensis*
17. Ratio of the length of basal part to terminal part 0.7 – 0.8 *P. jiarhaiensis*
Ratio of the length of basal part to terminal part 3.0 – 3.2 *P. luanchuanensis*

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中国跃蛎属（石蛎目，石蛎科）一新种记述

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摘 要 记述采自江苏南京跃蛎属 1 新种, 老山跃蛎 *Pedetontinus laoshanensis* sp. nov., 通过扩增其线粒体 CO I 基因, 与中国已知跃蛎属其它物种进行遗传距离比较, 并构建系统发生树, 发现其与天目跃蛎 *P. tianmuensis* 和武当跃蛎较为接近, 这也更加支持了基于形态证据的系统发生关系。并提供了跃蛎属已知 19 种的检索表。

老山跃蛎, 新种 *Pedetontinus laoshanensis* sp. nov. (图 1 ~ 17)

体长 7.0 ~ 9.0 mm, 复眼长/宽为 0.99 ~ 1.00, 中连线/长为 0.50 ~ 0.55, 复眼近中连线具浅色白斑, 阳基侧突为 1 + 6 型, 阳茎基节/端节为 2.4, 阳茎和阳基侧突位于第 IX 肢基片的 1/2, 产卵管初级型, 其长略超第 IX 腹节的刺突端刺。前产卵管, 分 49 ~ 50 节, 后产卵管, 分 47 ~ 50 节。该物种

关键词 石蛎目, 跃蛎属, 新种, 中国, CO I 基因.

中图分类号 Q969.121

CO I 基因信息见 GenBank No. EU699175 ~ EU 699177。

新种与泰山跃蛎 *P. songi*、天目跃蛎 *P. tianmuensis* 和武当跃蛎 *P. wudangensis* 相似, 但复眼中连线/长、阳茎基节/端节、阳茎和阳基侧突位置、产卵管分节具明显区别。

基于 CO I 基因序列构建 BI、MP 和 NJ 系统发生树均有效证明老山跃蛎 *P. laoshanensis* 为单系群。基于 K2p 模型, 新种与中国跃蛎属其它物种的遗传距离比较, 与天目跃蛎 *P. tianmuensis* 遗传距离最小 9.7%, 与栾川跃蛎 *P. luanchuanensis* 遗传距离最大 12.1%。结合形态和分子数据证明该物种的有效性。

正模 ♂, 江苏南京老山 (32°07'N, 118°36'E; 海拔 145 m), 2004-08-01, 李鹏、张加勇采, 编号 ZJNUSB050。

词源: 新种种名源自采集地地名。

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